

APPENDIX A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Number	:	09/646,347	Confirmation No.:	8741
Applicant	:	Marilyn E. KARAMAN, <i>et al.</i>		
Filed	:	January 4, 2001		
Title	:	METHOD OF WATER PURIFICATION		
TC/Art Unit	:	1724		
Examiner:	:	Ivars C. CINTINS		
Docket No.	:	63213.000003		
Customer No.	:	21967		

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Declaration of Heriberto Alejandro Bustamante under 37 C.F.R. §1.132

Sir:

I, Heriberto Alejandro Bustamante, do hereby state, that:

1. I reside at 1 Barjadda Avenue, Sylvania, NSW 2224 Australia. I received a PhD degree in Mineral Processing from Imperial College (London, England). My professional experience is detailed on my resume, attached hereto.
2. I am one of the inventors of the subject matter of US Patent Application No. 09/646347, the "present application" filed in the United States of America on 4 January 2001.
3. The present application relates to a method for the removal of biological species such as *Cryptosporidium* oocysts from water using aluminum based media which contains surface Al-OH groups.
4. According to the specification of the present application at page 5 lines 5-12, "Particulate alumina, such as powdered and granulated forms, provide an increased surface area per volume.

Powdered and granular alumina is readily available in different size ranges. Another particulate size range is from about 1.5mm to about 0.5mm. Yet another particle size contemplated by the present invention is from about 0.5mm to about 0.05mm".

5. Batch tests and pilot plant tests (details of which are provided below) were carried out for three different alumina size fractions by the Applicant. Batch tests were performed to determine the effect of particle size on the removal of *Cryptosporidium* oocysts by hydrated alumina (in bed filter material having different depths).

6. The Applicant found that an aluminum based medium which contains surface Al-OH groups, e.g. hydrated alumina (Al_2O_3), was most effective for removing *Cryptosporidium* oocysts from water if the particle size is below 1mm. The Applicant found that hydrated alumina (Al_2O_3) having a medium particle size of less than 1.0mm and more than 0.5mm was the most efficient for use in the pilot plant tests.

MATERIALS

Alumina Particle Size Fractions

7. Particle sizes of alumina assessed:

1. Large particle size of alumina (LPSA) – This size fraction comprised particles that were smaller than 4mm and larger than 2mm.
2. Medium particle size of alumina (MPSA) – This size fraction comprised particles that were smaller than 1mm and larger than 0.5mm.
3. Fine particle size of alumina (FPSA) – This size fraction comprised particles that were smaller than 0.2 mm and larger than 0.1mm.

Water

8. Sydney's tap water was used in all experiments. The tap water was spiked with various amounts of gamma-inactivated *Cryptosporidium* oocysts.

Sand

9. Sand was used as control (blank) filter material.

METHODS

***Cryptosporidium* Oocysts Removal Tests**

10. Two types of removal tests were assessed in the study, namely, batch tests and continuous tests. The batch tests were designed to identify a particle size that would be most effective for use in the pilot plant test. The pilot plant tests were designed to maximize the removal of *Cryptosporidium* oocysts from a substantial volume of water.

Batch Tests

Procedure to run batch tests.

11. Batch tests were carried out in a glass column (28mm x 18cm). The glass column had a 90 micron sintered filter at the bottom to hold the particles. Before each batch test the glass column was thoroughly rinsed with tap water. In addition, the column was further cleaned in an ultrasound bath after every third experiment.

12. The glass column was loaded with hydrated alumina particles of fine, medium, or large size. The amount of hydrated alumina particles in the glass column differed depending on the particle size being tested. The loaded column was filled with water to around 2cm above the hydrated alumina particles to prevent working with a "dry" bed of particles.

13. A 5 μ L aliquot containing 10^5 oocysts/mL was added to 30mL of tap water to obtain 500 oocysts/30mL. The dispersion was vortexed for around 30 seconds to ensure maximum homogenization and promote de-clumping of the oocysts.

14. All 30mL of tap water containing 500 *Cryptosporidium* oocysts was added to the glass column for one minute after which the water was allowed to flow out. The typical filtration time from the bed of particles was about 2 minutes. After the water exited the column, the bed of hydrated alumina particles was rinsed with 3 portions of 5 mL of *Cryptosporidium* free tap

water. This procedure was designed to ensure that any "loose" *Cryptosporidium* oocysts that may be mechanically trapped in the column were released.

15. All of the rinsing water was collected and combined with the initial 30mL. The total volume of water was approximately 200mL. The number of *Cryptosporidium* oocysts was determined in each 200mL water sample. This procedure was adopted to avoid subsampling of the filtered water and minimise analytical error. Sand (or in some cases glass spheres) was used as a control material (i.e., a blank) for each of the various particle sizes of hydrated alumina.

Continuous Tests

16. Continuous tests were carried out in a small pilot plant. The pilot plant comprised (i) a 400 L PVC tank, (ii) a variable flow pump and (iii) a glass column (50cm x 4cm) comprising medium size alumina particles (MSPA) or sand. The height of the MSPA or sand bed in the column was about 32cm.

Pilot plant operation

17. The glass column was prepared by placing a 90 micron sintered filter at the bottom and then loading the column to a bed height of about 32 cm with MSPA or sand. Water was added to the column to about 5cm above the level of the bed of particles. The 5cm level of water above the bed of particles was maintained constant to avoid working with a "dry" bed.

18. *Cryptosporidium* oocysts were added to the 400L PVC tank with tap water. The water in the tank was continuously stirred to minimize settling of the *Cryptosporidium* oocysts. The *Cryptosporidium* oocysts and water were then pumped to the glass column.

19. The average number of *Cryptosporidium* oocysts in the water fed to the glass column was approximately 3,000 – 3,500 per litre. Under these conditions it was possible to run the pilot plant for 20 days.

20. Sand was used as the control (blank) filter material for the pilot plant tests. The particle size range of the sand used as control was similar to that of MSPA, namely the sand particles were smaller than 1 mm and larger than 0.5mm.

Results for batch tests

21. Table 1 shows the effect of particle size and depth of the filter bed on the removal of *Cryptosporidium*:

TABLE 1

Particle Size Fraction	Depth of particle bed (mm)	Oocyst Removal (%)
Fine particles (less than 200 μ m more than 100 μ m)		
	3	95
	5	96
	10	98
Medium size particles (less than 1.0mm more than 0.5mm)		
	50	82
	100	89
	150	96
Large size particles (less than 4mm more than 2mm)		
	50	21
	100	41
	150	58

22. Thus, it is apparent, with particles having sizes in the range of about 1mm or less, oocyst removal of more than 80% and even up to 98% can be reached. For particles larger than 2mm,

the percentage removal is much smaller. Particles smaller than 200 μm showed good oocyst removal, however fine particles are not suitable for application in the pilot plant because the filtration rate is low and would require a pressurized filtration system rather than gravity alone.

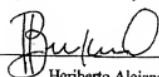
Results for pilot plant operation

23. The operation of the pilot plant demonstrated that particles having sizes in a range of about less than 1mm and more than 0.5mm are workable in a gravity fed filtration system, and enable a reasonable reduction in oocysts. Tests carried out using large particle size alumina particles resulted in less than 10% removal of *Cryptosporidium* oocysts.

24. The average number of oocysts in the column of medium sized alumina particles (less than 1.0mm and more than 0.5mm) was approximately 3,000 - 3,500 oocysts/l. Under these conditions it was possible to continuously treat the oocyst-containing water for 20 days. Over the 20 days the column was fed with around 9,000,000 oocysts in total. During this time the oocysts removal by the bed of medium size alumina particles was consistently between 2.5 and 3.5 log removal. By comparison the removal of oocysts by medium size sand particles only reached 0.6 log removal in a period of one week and its use was therefore discontinued.

25. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine and imprisonment, or both, under 18 U.S.C. §1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed



Heriberto Alejandro Bustamante

Place

Sydney (Australia)

Date

7 February 2006

APPENDIX B

CURRICULUM VITAE

NAME: Dr Heri A Bustamante

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PRESENT POSITION: Project Manager

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EDUCATION AND QUALIFICATIONS

PhD (Mineral Processing) Imperial College (London, England) 1976 -1979.

BSc (Pharmaceutical Chemistry) University of Chile (Chile) 1967 – 1972

CAREER DETAILS

Sustainability Division Sydney Water (previously with SWC's Australian Water Technologies)
1996-todate

Project Manager

Duties: Identify/develop technology opportunities that can lead to technology commercialisation or can be introduced to the water industry. Develop, direct and manage projects with internal and external clients. Identify, recommend areas that need consultancy investigations and R&D, organise team to carry out the projects.

THE UNIVERSITY OF NEW SOUTH WALES September 1993 - June 1996
Department of Water Engineering (Centre for Wastewater Treatment)

Program Manager Physico-Chemical Processes (also Adjunct Senior Lecturer)

Duties: Develop, lead, manage and resource R&D projects with granting organisations such as CRC Waste Management and Pollution Control, Landcare etc.

BP RESEARCH, SUNBURY-ON-THAMES, ENGLAND 1984 - Dec 1992

Senior Chemist, Environmental Engineering Team

Duties: Develop/Assess and introduce emerging environmental technologies for various business units in order to mitigate their environmental costs. Identify technology providers (European universities and companies) and negotiate both technologies testing and technology transfer.

Technologist, Mineral Processing Branch 1984 – 1990

Duties: Design, develop and assess novel technologies for introduction into the BP Minerals business in order to treat difficult ores more efficiently than with conventional technologies.

CSIRO DIVISION OF MINERAL CHEMISTRY, AUSTRALIA 1980 - 1984

Research Scientist

UNIVERSITY OF CONCEPCION, CHILE 1973-1976

Lecturer

PROFESSIONAL MEMBERSHIPS

Member of The Institute of Materials, Minerals and Mining (England)
Chartered Engineer (UK Engineering Council) Registration No 380060

MEMBERSHIP OF COMMITTEES

BP's representative at the Atomic Energy Authority / Water Research centre's Effluent Processing Club (EPC).
Member of the Working Group of Chemical Process Panel in EPC (Between Jan 1991-Dec 1992).

PUBLICATIONS

Surface Chemistry of Minerals

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9. **Bustamante H and Shergold H L**, The Surface Chemistry and Flotation of Zinc Oxide Minerals I. Flotation with Dodecylamine, Trans Inst Min Metall, 1983, 92, C201-C208.
10. **Bustamante H and Shergold H L**, The Surface Chemistry and Flotation of Zinc Oxide Minerals II. Flotation with Chelating Agents, Trans Inst Min Metall, 1983, 92, C208-C215.
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13. **Bustamante H and Rutter P R**, Flocculation of Heterodisperse Suspensions of Coal, Chem Eng Sci, 1987, 43(4), 809-821.

Water/Wastewater Treatment Related Papers

14. Waite T D, **Bustamante H**, Anderson N and Brungs M, Investigations into Management Options fro Water Treatment Plant Residuals in Australia, The Management of Water and Wastewater Solids for the 21st Century, WEF Specialty Conference Series Proceedings, pages 4/49-4/60, June 19-22, 1994, Washington (USA).
15. Waite T D and **Bustamante H**, Developments in Management of Water and Wastewater Treatment Plants Sludges: Sludge Characterisation, Dewatering and Reuse, Proceedings of International Conference on Asian Water Technology 94, 311-322, (November 22-24 1994), Singapore
16. **Bustamante H** and Waite T D, New Possibilities for Dewatering and Recycle of Water Treatment Plants Residuals, Proceedings of CSIRO-UNIDO International Workshop on Modern Techniques in Water and Wastewater Treatment, (Eds L O Kolarik and A J Priestley, CSIRO Publishing, Melbourne) 163-169, 1995,

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22. **Bustamante H** Removal of Flotation Collectors from Wastewater, Proceedings of 2nd Conference on Cleaner Technologies for the Mineral Industry, Santiago (Chile), May 1996, 30-36.
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